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DAILY POSTTRAINING EXPOSURE TO PULSED MAGNETIC FIELDS THAT EVOKE MORPHINE-LIKE ANALGESIA AFFECTS CONSEQUENT MOTIVATION BUT NOT PROFICIENCY IN MAZE LEARNING IN RATS

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ABSTRACT

Male rats were exposed to sham field conditions or to pulsed (burst firing pattern for 1 s every 3 s) magnetic fields (1–4 μT) for either 5 min or 30 min either immediately or after a 30 min delay following 8 daily training sessions in a maze. The maze was automated to record the proficiency of spatial learning and memory as well as response (lever press)/reward ratios. The strongest effect (approximately 40% of the variance) over the sessions occurred when the rats were exposed for 30 min to the field immediately after the training. Analyses of the data suggested that the reduced rate of acquisition was due primarily to the enhanced value of the reward rather than to interference with the consolidation of the spatial pattern.

INTRODUCTION

The consolidation of experience progresses through stages of variable lability that are vulnerable to different types of interference and manipulation (1). Gross cerebral mechanical impact, such as a concussion (with or without unconsciousness) and electroconvulsive shock, are known to permanently retard the consolidation and hence

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reconstruction of the experience that occurred during the previous 30 min (2,3). The hypothesis that exposures to extremely low frequency (ELF) electromagnetic fields following a learning episode should modify subsequent behavior has been considered intermittently (4). The fact that only 1 s of high-frequency stimulation can evoke spine growth on neuronal dendrites of hippocampal structures within 10–15 min (5) enhances the palatability of this hypothesis.

The measurement of learning is an inference that is based on the animal's performance. In general, performance is influenced by both the state of the consolidation of the memory (habit strength) and the correlative motivational variables. Fleming, Persinger, and Koren (6) reported that single or repeated 30-min exposures to a particular pulsed pattern of weak magnetic fields could increase the nociceptive thresholds of rats. The present experiment was designed to differentiate the consequences of postsessional exposure to a pulsed magnetic field upon the positional (spatial) or motivational (affective) components of memory.

The working hypothesis has assumed that a brief exposure to 1 s on/3 s off magnetic fields whose temporal structure simulates burst firing within the limbic system elevates endogenous analgesics. The class of analgesics is not likely to involve mu enkephalin since the effects of the field were not ameliorated by preinjection with naloxone (6). However, there is converging evidence (7,8) that this domain of weak (1 ηT to 1 mT), extremely low frequency magnetic fields may affect the corticotropin-releasing factor (CRF) systems and the longer neuropeptide sequences to which both adrenal corticotropic hormone (ACTH) and the endorphins belong. In general, experimental injection of endorphins has interfered with the learning and the memory of a variety of tasks (9).

If the primary measure of learning or memory is efficiency (as defined by fewer errors or fewer repetitions of previous nonrewarded behaviors) and the primary measure of motivation is speed (as defined by the time required to achieve a level of efficiency), then inferences for both constructs must be present within the same experimental paradigm in order to differentiate their potential effects upon the observed behavior. The possibility that the field treatment could increase the relative potency of the reward can be inferred by the numbers of additional level presses that occur following the delivery of the single reward.

Our work (10) has employed an automated (PC-driven) maze that has four arms; a lever and pellet dispenser is present at the distal portion of each arm. The rat must learn a particular sequence of arm entries in order to obtain, without error, a reward following each bar press. Only one reward is associated with a lever press in each arm during a sequence despite the number of lever presses.

If postsessional exposures to this pattern of magnetic field simulate the effects of exogenous injections of endorphins, then rats that receive this treatment should exhibit fewer successful completions (trials) of the maze. If the poorer proficiency is due to interference with the consolidation of the correct sequence of ambulatory movements, then the rats should exhibit more reentries of previously rewarded arms before the trial is completed. If the relative deficits are due to motivational factors, then the rats should exhibit slower movements during the execution. Finally, if the effects of the field exposures following the daily training increase the reward value of the reinforcement area or the food pellets, then these rats should demonstrate an increase in the number of lever presses even though only one reward is delivered per arm, per successful sequence.

MATERIALS AND METHODS

In three experiments a total of 36 male, 80–100-day-old Wistar albino rats served as subjects. They were obtained from Charles River (Quebec). During the first 2 weeks after delivery, the rats were maintained in groups of 3 per cage within standard colony housing. The temperature was controlled between 20 and 21°C and the light-dark cycle was 12:12 (night phase began at 19:30 h). Food and water were available *ad libitum*. The protocol for the experiment had been approved by the local animal care committee.

When the study began, rats were housed separately and gradually reduced to 85% for their free-feeding body weights. After several days of lever training to criterion (200 rewards in 30 min) in standard operant chambers, they were placed in an automated, four-arm maze (10). An operant box lever and pellet dispenser was located at the end of each arm. To obtain continuous reinforcement, the rat was required to learn that reentry into an arm and lever pressing would not deliver another 50 mg Noyes food pellet until each of the four levers in each of the four arms had been pressed sequentially. The pattern (order of arms) was always fixed.

Each rat was placed in the maze for 30 min once per day for 8 successive days. The number of successful rotations (trials), the time required to complete each trial, the mean number of reentries into a previously rewarded arm per trial, and the mean number of responses per reinforcement were recorded automatically by computer for each rat on each day (30-min session). To facilitate analyses, the data for each of two successive days were combined into blocks such that there were a total of four blocks.

In experiment I, 6 rats were exposed for 30 min, immediately after removal from the daily maze training, to a pulsed magnetic field that was generated by the equipment (6,11) and within the setting (another room) that has been employed previously. In brief, a burst-firing magnetic field, whose strength within the exposure area ranged between 1 to 4 μT (10–40 mG), was presented for 1 s once every 3 s through two solenoids. The reference group ($n = 6$) was exposed to the same apparatus except the computer was not activated. In experiment II, 6 rats were exposed to the field for 5 min while another 6 rats served as the sham field–exposed reference group. Because there were no significant differences between the two control groups and in order to facilitate analyses, the experiments were combined.

For experiment III, which was initiated and concluded 1 year later, 4 rats were exposed to one of 3 conditions. The reduced sample was employed because if the effect size for the alterations in response/reward ratios was as robust as noted in experiments I and II, this sample size would be sufficient. Four rats were exposed to the sham field condition and another 4 rats were exposed to the pulsed field for 30 min immediately after the daily maze training. For the third treatment, an additional 4 rats were exposed to the pulsed field for 30 min but only after 30 min had elapsed since removal from the maze. During the intervening period, these rats were housed in their home cages.

The numbers of successful trials, the numbers of responses per reward, the average numbers of reentries per arm, and the time required for each trial were loaded onto an alpha open VMS computer system. Analyses of variance, *post hoc* tests, and repeated measure analyses of variance (MANOVA) were completed by SPSS software.

RESULTS

The means and standard errors of the mean for the numbers of successful completions (trials) per session for each of the 4 blocks for rats that were exposed to either the sham field condition or to the pulsed magnetic field for either 5 min or 30 min immediately after each daily training period in the maze are shown in Table 1. Two-way analysis of variance (MANOVA) with one level repeated (blocks) and one level not repeated (treatment) demonstrated a marginally significant ($p = 0.05$) difference between the groups [$F(2,21) = 3.32$; $\varepsilon^2 = 24\%$]. In addition to the learning effect [$F(3,63) = 56.95$; $p < .001$], there was a statistically significant interaction between treatments and time [$F(6,63) = 2.87, p < .05$; $\varepsilon^2 = 21\%$). *Post hoc* analysis (contrasts of correlated t-tests and Tukey's set at $p < .05$) indicated that the major source of the effect was due to the fewer successful completions of the maze during the middle of the training period (blocks 2 and 3) for the group that had been exposed immediately after the training for 30 min to the pulsed fields, relative to the sham field–exposed group or to the group that was exposed to the pulsed field for only 5 min; they did not differ significantly from each other.

However the consequences (experiment III) of exposure for 30 min to the sham field or to the pulsed field for 30 min either immediately after the training or after a 30-min delay following daily training did not demonstrate any statistically significant main treatment effects [$F(2,9) = 1.50, p < .10$]. Although the expected influence of learning over blocks was apparent [$F(3,27) = 23.39, p < .001$], there was no significant interaction between treatment and blocks [$F(6,27) = 1.05, p < .05$].

The ratio of responses per reward in each arm demonstrated statistically significant [$F(2,21) = 4.46, p < .05$; $\varepsilon^2 = 30\%$] differences between the treatments as well as

Table 1. Means and Standard Errors of the Mean for the Number of Successful Rotatins per Block for Rats Exposed to Sham Fields or to Pulsed Magnetic Field After Each Maze Period for Either 5 min or 30 min or for 30 min Either Immediately After or After a 30-min Delay

	Experiments I and II (combined)					
			Pulsed magnetic field			
	Sham field		30 min		5 min	
Block	M	SEM	M	SEM	M	SEM
1	5.7	1.0	4.2	0.6	2.6	1.5
2	21.5	4.8	4.6	1.2	14.8	5.7
3	40.4	6.4[a]	9.0	3.7[b]	31.2	7.9
4	56.5	8.5	26.2	7.4	54.0	10.4
	Experiment III					
	Sham field		30 min (delay)		30 min (immed.)	
Block	M	SEM	M	SEM	M	SEM
1	4.0	0.0	5.1	0.2	3.8	0.9
2	17.6	5.2	26.4	8.6	8.4	1.0
3	30.3	11.7	44.9	15.9	13.3	5.2
4	42.4	7.4	54.0	15.6	41.9	8.1

[a] vs. [b] *post hoc* $p < .05$.

Table 2. Means and Standard Errors of the Mean for the Ratio of Bar Presses to Rewards (mean of all four levers for each of four arms) During Maze Acquisition for Rats that Were Exposed to Sham Conditions or to the Pulsed Field for 30 min Either Immediately or Delayed by 30 min

Experiments I and II						
			Pulsed magnetic field			
	Sham field		30 min		5 min	
Block	M	SEM	M	SEM	M	SEM
1	11.6	1.1	13.4	1.3	13.0	0.9
2	8.4[a]	0.7	18.0[b]	3.9	10.5	2.0
3	3.1[a]	0.7	6.6[b]	1.6	3.1	0.8
4	2.3	0.6	3.2	0.6	7.2	4.5
Experiment III						
	Sham field		30 min (immed.)		30 min (delay)	
Block	M	SEM	M	SEM	M	SEM
1	10.9	0.6	12.9	3.1	9.3	0.9
2	8.5	1.3	11.5	1.1	7.2	1.9
3	5.5	2.1	15.0[a]	3.7	4.8[a]	2.1
4	2.9	0.6	2.9	0.4	3.7	1.6

[a] vs. [b] *post hoc* $p < .05$.

a significant [$F(6,63) = 4.44, p = .001$; $\varepsilon^2 = 30\%$] interaction between treatments and blocks. The learning effect was also statistically significant [$F(3,63) = 21.60, p < .001$; $\varepsilon^2 = 50\%$]. *Post hoc* analysis of the main effect indicated that the group that had been exposed for 30 min to the pulsed field emitted about twice the number of lever presses per reward than did the sham field group or the group that had been exposed to the

Table 3. Mean Time (in min) for the Completion of Trials (a single bar press at each of four arms) for Experiments I–II and III

Experiments I and II (combined)						
			Pulsed magnetic field			
	Sham field		30 min		5 min	
Block	M	SEM	M	SEM	M	SEM
1	5.5	1.4	5.0	0.7	5.0	1.0
2	1.9	0.5	7.6	2.3	3.6	1.0
3	0.6	0.2	1.4	0.9	0.7	0.7
4	0.5	0.5	0.7	0.3	2.4	1.7
Experiment III						
	Sham field		Immed.		Delayed	
Block	M	SEM	M	SEM	M	SEM
1	9.1	1.2	7.7	1.7	6.2	0.3
2	4.1	1.2	5.2	0.3	3.3	0.8
3	3.5	1.6	6.9	1.7	2.7	1.1
4	1.9	0.3	1.4	0.2	2.0	1.5

field for 5 min. The primary source of the interaction occurred during the middle portion of the 8 days of training.

Two-way analysis of variance (experiment III) verified the effects of the immediate exposure for 30 min to the pulsed fields with respect to the number of lever responses per food pellet at the reward sites of the maze. The rats that had been exposed to this condition displayed significantly higher ratios of responding [$F(2,9) = 4.38, p < .05$; $\varepsilon^2 = 49\%$) than did either the sham field–exposed group or the group that received the identical field exposure (but delayed by 30 min). The latter two groups did not differ significantly from each other (Tukey's set at $p < .05$).

The mean time (in minutes) required to complete each successive sequence (response-contingent reward in each of 4 successively different arms) for the three experiments is shown in Table 3. The statistically significant [$F(2,21) = 5.24, p = .01$; $\varepsilon^2 = 33\%$] treatment difference for the first 2 experiments and the interaction between blocks and treatment [$F(6,63) = 5.06, p < .001$; $\varepsilon^2 = 33\%$] was (according to *post hoc* Tukey's and correlated *t*-tests) primarily due to the slower completion times for the rats that were exposed immediately after each daily session to the pulsed field relative to the other 2 groups that did not differ significantly from each other. Neither the main treatment effect [$F(2,9) = 1.52$] nor the interaction between treatments and time [$F(6,27) = 1.89$; $\varepsilon^2 = 30\%$] was significant statistically ($p > .05$) for experiment III. It may be important that the latter approached statistical significance ($p = 0.10$) and was due in large part to the slower completion times during the middle of the learning curve for the rats that were exposed to the field immediately after the training relative to the delayed group.

Repeated measure analyses of variance for the 2 blocks of experiments did not demonstrate any statistically significant differences between the groups with respect to the number of times the rats reentered previously rewarded arms on each trial [$F(2,21) = 1.51$; $F(2,9) = 1.52, p > .05$, for experiments I–II and experiment III, respectively]. The interactions between treatments and sessions were also not statistically significant [$F(6,63) = 1.29$; $F(6,27) = 1.89, p > .05$, respectively]. For the first block of sessions, the grand means and standard deviations (in parentheses) for the numbers of reentries into the arms for experiments I–II and experiment III were 6.8 (3.7) and 7.7 (2.7), respectively. These values for the last block (4) of sessions were 1.7 (0.7) and 1.8 (0.9), respectively.

DISCUSSION

Attempts to determine the vectorial (direction and magnitude) effects of both static and time-varying and both strong (T) and weak (μT to mT) magnetic fields upon learning and memory have emphasized testing *after* the field exposures (e.g., 12,13). Exposure to weak, time-varying fields during (14) or immediately after the display of the behavior (and the peak concurrent neuroelectrical activity) has been pursued less frequently. However, when the former is evaluated, significant alterations in the rate of learning of a task that requires spatial memory have been shown to occur following very brief exposures to mT fields (14).

As discussed frequently by the Ossenkopp and Kavaliers research group (14,15), opioid systems within the brain have been implicated as prominent mediators of both learning and memory. Opioid agonists impair while opioid antagonists facilitate behaviors that have been inferred to reflect the memory of spatial patterns. Exposures to 60-Hz magnetic fields appear to attenuate endogenous opioid activity because the

presentation of these energies has been reported to enhance the acquisition of a spatial maze by rodents (14).

The present study involved exposures immediately (for 30 min) after daily training periods to a burst-firing, pulsed field that has been shown to elevate nociceptive thresholds in rats and to simulate the effects of 1 mg/kg of morphine (6). The equipment and the exposure parameters were designed to differentiate the effects of "electrical disruption" upon consolidation from motivational (affective or reward) factors. In both blocks of experiments, the strongest and most consistent effect was the increased number of responses per reward on subsequent days when the rats were exposed to this pulsed field immediately during the 30-min period following daily training.

This observation would be consistent with the hypothesis that enhancement of the affective components of a transient, reverberating pathway during the period of neuroelectrical consolidation can specifically enhance this domain or dimension of the memory trace (16). At least 2 major domains of memory, a primary spatial representation and a primary affective component of this representation, have been considered hypothetical constructs. However, there is one pivotal structure whose intrinsic organization would accommodate the demands of these constructs and serve as a neuroanatomical correlate.

The cingulate cortex, which contains substantial densities of opioid receptors, was an important emergent structure of the mammalian brain (17). Whereas the posterior portion of this cortex receives primary information from the hippocampal formation and the parietotemporal cortices, the anterior portion of the cingulate receives primary information from the amygdala and mediodorsal thalamus. Damage to the caudal portion of this structure or its inputs is associated with navigational impairment and deficits for spatial memory, while damage to the anterior region of the cingulate encourages distractibility and indifference to pain (18).

The counterargument that the increased lever response/reward ratios emitted by these rats simply reflected perseveration is not likely. Perseveration is usually persistent and is not (usually) expected to attenuate to reference levels. Both observations of the rats and the data patterns indicated that the groups that had been exposed for 30 min every day immediately after the trials remained around the reward area for longer periods and hence displayed more responses despite the delivery of only one food pellet. If the fewer numbers of successful completions had been artifactually created by more random activity or erroneous entry into arms that were (during that trial) no longer associated with reward, then group differences for the number of reentries per arm would have been statistically significant; this effect did not emerge.

The validity and reliability of these results, despite the small sample, is indicated by the similarity of the effect size for the two groups (in two separate experiments) that had been exposed for 30 min to the field immediately after the training. The effect size of this particular magnetic field treatment was similar to its influence on the nociceptive threshold for electric current (6). The fact that the effect was not apparent if a 30-min delay occurred before the exposure to the field or if the exposure occurred for only 5 min immediately after the daily trials indicates the specificity of the phenomenon.

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